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Comparison of three sample preparation methods on the recovery of volatiles from taheebo (*Tabebuia impetiginosa* Martius ex DC)

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ABSTRACT: The volatile constituents of taheebo (the dried inner bark of *Tabebuia impetiginosa* Martius ex DC) were isolated by three methods: solvent-assisted flavour evaporation (SAFE); steam distillation under reduced pressure, followed by continuous liquid–liquid extraction (DRP-LLE); and high-flow dynamic headspace sampling (including closed-loop stripping) (DHS). The extracts were analysed qualitatively and quantitatively by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS). The masses of total volatile components recovered from 50 g taheebo by SAFE, DRP-LLE and DHS were 26.3 ± 2.0, 19.4 ± 1.0 and 1.2 ± 0.2 mg, respectively. The major constituents isolated with the SAFE method were 4-methoxyphenol (121.65 μg/g), 4-methoxybenzyl alcohol (96.49 μg/g), 1,2-propanediol (92.95 μg/g) and 4-methoxybenzaldehyde (31.33 μg/g). The DRP-LLC method yielded 4-methoxybenzaldehyde (64.54 μg/g), 4-methoxyphenol (42.30 μg/g), 5-(2-propenyl)-1,2,3-trimethoxybenzene (elemicin; 41.22 μg/g), and 1-methoxy-4-(1*E*)-1-propenylbenzene (*trans*-anethole; 39.16 μg/g) as major volatiles, while the main compounds recovered with the DHS method were 4-methoxybenzaldehyde (2.61 μg/g), 4-methoxyphenol (2.10 μg/g), 2-methyl-5-(1-methylethenyl)-2-cyclohexen-1-one (carvone; 1.71 μg/g) and 3,7-dimethyl-1,6-octadien-3-ol (linalool; 1.64 μg/g). Copyright © 2004 John Wiley & Sons, Ltd.

KEY WORDS: volatile components; solvent-assisted flavour evaporation (SAFE); dynamic headspace sampling (DHS); pau d'arco

Introduction

The bark of various South and Central American *Tabebuia* species is sold under the name of 'taheebo', 'pau d'arco' or 'lapacho'. The material is reported to possess astringent, antiinflammatory, antibacterial, antifungal, diuretic and laxative properties. Major constituents in methanol extracts of *Tabebuia* spp. have been reported several times. They included furanonaphthoquinones,[1–3] quinones,[4] naphthoquinones,[5] benzoic acid, benzaldehyde derivatives,[6] cyclopentene dialdehydes[7] and flavonoids.[8] Many studies have investigated the biological and pharmacological effects of *Tabebuia* spp. extracts and their isolated compounds.[9–14] To our knowledge, the only volatile constituents of taheebo that have been reported to date are 3,4-dimethoxybenzaldehyde, 4-hydroxy-3-methoxybenzaldehyde (vanillin) and 4-methoxybenzaldehyde.[6] The volatile chemical composition of the extracts isolated from plants depends on the type of sample preparation technique employed. Chemical transformations such as hydrolysis can occur during steam distillation. Chaintreau reviewed the development of simultaneous distillation–extraction and compared this technique with other sample preparation methods to illustrate its capabilities and limitations.[15] Practical guidelines for its use, along with ways to minimize artifact formation, were also discussed. Solvent-assisted flavour evaporation (SAFE) is the one of the most versatile methods used for the isolation of food and plant volatile constituents.[16] This technique allows the fast and careful isolation of volatiles from complex food matrices under very mild conditions. High-flow dynamic headspace sampling, when combined with the addition of excess sodium sulphate, leads to the identification of highly water-soluble volatiles that are not effectively isolated by conventional sample preparation methods.[17] Advantages and disadvantages of dynamic headspace sampling and simultaneous distillation–extraction as methods for isolating food volatiles were reported by Buttery and Ling.[18] Mayer *et al.*[19] compared the recovery of important tomato flavour constituents using three sample preparation methods. The dynamic headspace method (combined with the addition of saturated calcium chloride solution to the tomato sample) gave good recovery of most of the compounds and performed better than the SAFE method for constituents such as phenylacetaldehyde (64%

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vs. 26% recovery), (*E*,*Z*)/(*E*,*E*)-2,4-decadienal (65% vs. 34%), β-damascenone (65% vs. 28%) and β-ionone (49% vs. 18%). With the exception of the compounds noted previously, the SAFE method gave recoveries similar to the dynamic headspace method and better recovery of water-soluble compounds such as 3-methylbutyric acid (83% vs. 28% recovery), 2-phenylethanol (69% vs. 29%) and 4-hydroxy-2,5-dimethyl-3-(2*H*)-furanone (23% vs. 5%). In this study, the volatile constituents of the dried inner bark of *T. impetiginosa* were isolated by three different sample preparation techniques: solvent-assisted flavour evaporation (SAFE); steam distillation under reduced pressure followed by continuous liquid–liquid extraction (DRP-LLC); and high-flow dynamic headspace sampling (DHS). The recovery and composition of the isolated volatiles were determined by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS).

Experimental

Plant Material

Taheebo was obtained from a local herb store (Berkeley, CA).

Isolation of Volatile Extracts

Solvent-assisted Flavour Evaporation (SAFE Method)

Taheebo (50 g) was extracted with 200 ml dichloromethane (Burdick & Jackson, Muskegon, MI) for 12 h at room temperature. The filtered extract was added to the dropping funnel of the SAFE apparatus, which was heated to 40 °C with a circulating waterbath. The distillation flask (500 ml) was heated to 40 °C in a waterbath. The receiving flask for the distillate and the safety-cooling trap of the SAFE apparatus were cooled with liquid nitrogen. The SAFE apparatus was connected to a high-vacuum pump (<0.01 Pa) and then the extract in the dropping funnel was added in small aliquots into the distillation flask over a period of 20 min. The frozen distillate was thawed at room temperature. The extract was dried over anhydrous sodium sulphate and concentrated to about 0.6 ml using a Vigreux column (15 × 1 cm) and waterbath at 40–50 °C.

Steam Distillation under Reduced Pressure Followed by Continuous Liquid–Liquid Extraction (DRP-LLE Method)

Taheebo (50 g) was placed in a 3 l round-bottomed flask with 1 l deionized water. The solution was steam-distilled at 55 °C for 3 h under reduced pressure (95 mmHg). The distillate (900 ml) was subjected to continuous liquid–liquid extraction for 6 h using 100 ml dichloromethane. After drying the extract over anhydrous sodium sulphate, the solvent was removed in a rotary flash evaporator (Yamato Sci., Tokyo, Japan). The distillation was stopped when the volume of extract was reduced to approximately 0.6 ml.

High-flow Dynamic Headspace Sampling (DHS Method)

Taheebo (50 g) was placed into a 1 l round-bottomed flask along with 150 ml purified water (Milli-Q Plus, Millipore Corporation, Bedford, MA) and 81 g NaCl (previously heated to 150 °C to remove volatiles). The flask was fitted with a Pyrex head to allow the sweep gas to enter the top of the flask (via a Teflon tube) and exit from a side-arm through a Tenax trap [ca. 10 g Tenax (Alltech Associates, Deerfield, IL), fitted with ball-and-socket joints]. The system was purged with nitrogen (200–400 ml/min) for 2 min and immediately connected to an all-Teflon diaphram pump that recirculated nitrogen around the loop (closed-loop stripping) at 6 l/min for 3 h. The sample was continuously stirred during the sampling period with a magnetic stirrer. After sampling, the Tenax trap was removed and the volatiles were eluted with 60 ml freshly distilled diethyl ether containing *ca*. 0.001% ethyl antioxidant 330 [1,3,5-trimethyl-2,4,6-tris(3,5-di-tert-butyl-4-hydroxybenzyl)benzene]. The eluate was concentrated to about 0.6 ml using a warm waterbath at 40 °C and a Vigreux column (15 × 1 cm).

Determination of Total Volatile Constituents in Extracts

The extracts from each method were transferred into a vial. The distillation flask was washed with a minimum amount of dichloromethane or diethyl ether, and the washings were added to the vial. The solvents were carefully removed using a purified nitrogen stream or by microdistillation with a Vigreux column (15 × 1 cm) until the total volume was reduced to approximately 100 µl. The mass of extract was measured with an analytical balance. The extract was then analysed by GC, using a flame ionization detector (FID) to determine the percentage of total peak area of volatile components and solvent. The total mass of volatile components was calculated by multiplying the percentage representing the total peak area of components by the total mass of extract. Each experiment was repeated three times. The detector response to solvent was found to be linear over a range of 0.2–1.0 µl injected, with a R^2 value of 0.99. A Hewlett-Packard Model 6890 gas chromatograph, equipped with a

30 m × 0.25 mm i.d. (d_f = 0.25 μm) DB-WAX bonded-phase fused-silica capillary column (J&W Scientific, Folsom, CA) and a FID, was used for analysis of total volatile components in each extract. The linear velocity of the helium carrier gas was 30 cm/s at a split ratio of 1:20. The injector and detector temperatures were 250 °C. The oven temperature was programmed from 50 °C to 180 °C at 3 °C/min and held for 20 min at the final temperature.

Identification of Volatile Constituents Isolated from Taheebo

Volatile constituents obtained by SAFE, DRP-LLE and DHS were identified by comparison of the compound's Kováts index, I^{20} and mass spectrum with that of a reference standard.

Capillary Gas Chromatography

A Hewlett-Packard Model 5890 gas chromatograph, equipped with a 60 m × 0.32 mm i.d. (d_f = 0.25 μm) DB-1 bonded-phase fused-silica capillary column (J&W Scientific, Folsom, CA) and a FID, was used for determination of Kováts indices and concentrations. The injector and detector temperatures were 170 °C and 250 °C, respectively. The oven temperature was programmed from 35 °C (4 min isothermal) to 230 °C (held for 25 min at final temperature) at 2 °C/min. The linear velocity of the helium carrier gas was 36 cm/s (30 °C) at a split ratio of 1:20.

Capillary Gas Chromatography–Mass Spectrometry (GC–MS)

The system consisted of Hewlett-Packard 6890 gas chromatograph coupled to Hewlett-Packard 5973 quadrupole mass spectrometer (capillary direct interface). A 60 m × 0.25 mm i.d. (d_f = 0.25 μm) DB-1 bonded-phase fused-silica capillary column was used. Helium carrier gas was used at a column head pressure of 22 p.s.i. The oven temperature was programmed from 35 °C (4 min isothermal) to 220 °C (held for 15 min at final temperature) at 2 °C/min.

Results and Discussion

The total yields of volatile constituents from taheebo were 0.053 ± 0.004% (SAFE), 0.039 ± 0.002% (DRP-LLC) and 0.0024 ± 0.0003% (DHS) (w/w). The masses of total volatile constituents isolated by SAFE, DRP-LLE and DHS from the 50 g of dried inner bark of *T. impetiginosa* were 26.3 ± 2.0, 19.4 ± 1.0 and 1.2 ± 0.2 mg, respectively. The values are given as mean ± standard deviation (n = 3). The low yield of volatiles with the DHS method may be in part due to the fibrous nature of the bark. The bark was sampled as received. However, we suspect that the yield could be improved by increasing the surface area, e.g. by freezing the material with liquid nitrogen and then blending or grinding it to a fine powder. Table 1 shows volatile constituents identified in taheebo, along with their concentrations and Kováts indices on a DB-1 column. Among the 100 peaks detected, 56 (SAFE), 49 (DRP-LLC) and 58 (DHS) volatile constituents were identified from the three different isolation methods. The identified volatiles included aliphatic constituents, aromatic constituents, terpenoids, phenylpropenoids, and miscellaneous compounds. The major constituents were quite different, depending on the isolation method. The major constituents isolated with the SAFE method were 4-methoxyphenol (121.65 μg/g), 4-methoxybenzyl alcohol (96.49 μg/g), 1,2-propanediol (92.95 μg/g), 4-methoxybenzaldehyde (31.33 μg/g) and *trans*-anethole (23.98 μg/g). The DRP-LLE method yielded 4-methoxybenzaldehyde (64.54 mg/g), 4-methoxyphenol (42.30 μg/g), elemicin (41.22 mg/g), *trans*-anethole (39.16 μg/g), 4-methoxybenzyl alcohol (28.43 μg/g), carvone (25.04 μg/g) and linalool (18.05 μg/g) as the major volatiles. The main constituents recovered with the DHS method were 4-methoxybenzaldehyde (2.61 μg/g), 4-methoxyphenol (2.10 mg/g), carvone (1.71 μg/g), linalool (1.64 μg/g), 4-methoxybenzyl alcohol (1.54 μg/g) and elemicin (1.03 μg/g). The SAFE method recovered almost three times more 4-methoxyphenol and more than three times the amount of 4-methoxybenzyl alcohol than the DRP-LLE method. The very polar 1,2-propanediol was only isolated with the SAFE method. 1,2-Propanediol, the third most abundant constituent in the SAFE extract, is a synthetic GRAS constituent commonly used in consumer and food products such as deodorants, lotions, pharmaceuticals and sour cream products. In light of other reports of contamination of dietary supplements,[21,22] the presence of 1,2-propanediol, although relatively non-toxic, is troubling. The terpene hydrocarbons, *p*-cymene, limonene and α-bergamotene, had much better recovery with the SAFE method than the DRP-LLE method. 3,4-Dimethoxyphenol and 3,4-dimethoxybenzyl alcohol were only recovered with the SAFE method. In contrast, elemicin had a concentration 15 times higher with the DRP-LLE method than the SAFE method. Other compounds that had about two-fold or more higher recovery in the DRP-LLE method compared to the SAFE method included 2-methoxyphenol, linalool, 1,2-dimethoxybenzene, 1,4-dimethoxybenzene, 4-terpineol, 2-methoxy-4-methylphenol, α-terpineol, 4-methoxybenzaldehyde, carvone, cinnamaldehyde, *trans*-anethole, thymol, carvacrol, eugenol, methyl

Table 1. Concentrations of taheebo volatiles isolated by three sample preparation methods

Constituents	*KI*[a]	DRP-LLE		SAFE		DHS	
		KI[b]	Conc. (μg/g)	*KI*[b]	Conc. (μg/g)	*KI*[b]	Conc. (μg/g)
1,2-Propanediol	710	—	—	716	92.95	—	—
Toluene	748	—	—	—	—	745	0.01
Pentanol	744	—	—	—	—	747	0.05
Hexanal	772	772	0.73	768	0.94	771	0.22
Furfural	800	798	2.15	794	1.97	797	0.37
Ui[c]	—	—	—	—	—	802	0.54
(*E*)-2-Hexenal	822	—	—	—	—	822	0.02
Furfuryl alcohol	827	827	tr[d]	827	1.56	829	0.04
(*Z*)-3-Hexenol	834	—	—	—	—	836	0.03
Hexanol	848	852	0.63	846	1.58	852	0.08
2-Acetylfuran	876	—	—	—	—	875	0.02
Benzaldehyde	926	922	1.01	920	0.63	922	0.12
5-Methylfurfural	926	924	tr[d]	923	0.26	925	0.03
Phenol	957	964	4.27	961	4.16	963	0.48
6-Methyl-5-hepten-2-one	961	968	0.26	963	tr[d]	965	0.03
1-Octen-3-ol	962	964	tr[d]	965	0.09	968	0.02
Benzyl alcohol	1004	1002	0.98	1000	1.59	1002	0.13
p-Cymene	1010	1006	tr[d]	1006	0.43	1008	0.02
1,8-Cineole	1018	1014	0.31	1012	0.48	1014	0.09
Limonene	1020	1020	tr[d]	1015	2.13	1017	0.06
4-Methylphenol	1056	1053	tr[d]	1051	0.20	1041	0.03
trans-Linalool oxide	1056	1054	tr[d]	1052	0.29	1054	0.10
2-Methoxyphenol	1058	1056	4.48	1053	2.02	1055	0.27
cis-Linalool oxide	1070	1070	tr[d]	1068	0.50	1071	tr[d]
2-Phenylethanol	1081	1078	3.48	1076	3.02	1078	0.24
Linalool	1083	1083	18.05	1081	10.61	1083	1.64
Ui[c]	—	1104	18.27	1101	5.53	1103	1.74
1,2-Dimethoxybenzene	1111	1111	4.22	1109	1.46	1111	0.36
Camphor	1118	1123	tr[d]	1121	0.37	1122	0.03
Menthone	1130	1127	0.58	1125	0.48	1126	0.08
1,4-Dimethoxybenzene	1132	1130	6.31	1128	3.64	1130	0.32
Borneol	1147	—	—	1142	0.78	1142	0.11
Menthol	1155	1153	1.09	1151	0.99	1152	0.17
4-Terpineol	1159	1156	3.85	1154	1.59	1156	0.36
2-Methoxy-4-methylphenol	1164	1161	4.22	1159	1.25	1161	0.35
α-Terpineol	1170	1167	2.09	1165	0.84	1167	0.18
Estragole	1173	1171	8.20	1169	7.66	1171	0.32
3,4-Dimethylbenzene[c]	—	1174	tr[d]	1174	0.94	1175	0.06
4-Methoxyphenol	1185	1186	42.30	1187	121.65	1186	2.10
3,4-Dimethoxytoluene	1204	1198	tr[d]	1199	0.31	1201	0.04
4-Methoxybenzaldehyde	1211	1207	64.54	1206	31.33	1206	2.61
Carvone	1213	1209	25.04	1208	10.34	1209	1.71
Cinnamaldehyde	1227	1223	7.31	1222	2.66	1223	0.19
4-Methoxybenzyl alcohol	1244	1242	28.43	1244	96.49	1242	1.54
trans-Anethole	1259	1257	39.16	1255	23.98	1257	0.87
Thymol	1268	1269	1.28	1267	0.60	1275	0.08
Carvacrol	1277	1277	2.75	1275	0.77	1277	0.09
2,6-Dimethoxyphenol	1309	—	—	1293	1.14	1294	0.07
Eugenol	1327	1324	4.15	1322	2.28	1323	0.15
Pyrogallol	1341	—	—	1329	0.51	1329	0.07
Methyl 4-methoxybenzoate	1333	1336	0.49	1333	0.21	1336	0.03
Methyl cinnamate	1348	1344	0.74	1342	1.89	1343	0.02
O-Methyleugenol	1370	1369	0.88	1371	0.52	1368	0.04
3,4-Dimethoxyphenol	1384	—	—	1383	2.49	—	—
3,4-Dimethoxybenzaldehyde	1422	1423	5.40	1422	4.76	1423	0.19
α-Bergamotene	1432	—	—	1427	2.37	—	—
3,4-Dimethoxybenzyl alcohol	1450	—	—	1449	8.55	—	—
3,4-Dihydro-8-hydroxy-3-methylisocoumarin[e]	—	1484	5.78	1483	1.21	1484	0.03
Elemicin	1516	1517	41.22	1515	2.68	1516	1.03
Diethylphthalate	1550	1547	tr[d]	1545	0.16	1546	0.03
Caryophyllene oxide	1550	1560	1.47	1557	0.22	1558	0.02
Benzophenone	1566	1573	tr[d]	1576	0.32	1577	0.03

[a] *KI*, reference Kováts indices on DB-1 column.
[b] *KI*, experimental Kováts indices on DB-1 column.
[c] Ui unidentified constituents.
[d] tr, trace.
[e] tentatively identified.

Table 2. Structural distribution in the volatiles isolated using three different extraction methods from *T. impetiginosa*

Classification	Peak area (%)		
	DRP-LLC	SAFE	DHS
Aliphatic constituents	0.42	18.17	1.91
Aromatic constituents	40.92	53.41	34.47
Terpenoids	14.56	6.43	19.71
Phenylpropanoids	15.58	7.41	6.61
Miscellaneous	15.51	2.62	9.82

4-methoxybenzoate and caryophyllene oxide. Some constituents that were only found with the DHS method were toluene, pentanol, (*E*)-2-hexenal, (*Z*)-3-hexenol and 2-acetylfuran. The structural class distribution of the volatiles isolated from the three sample preparation methods is summarized in Table 2. The SAFE method had, by far, the highest proportion of aliphatic constituents [18.17% (SAFE) vs. 1.91% (DHS) and 0.42% (DRP-LLE)]. 1,2-Propanediol, which accounted for the majority (>93%) of the aliphatic constituents, was only detected with the SAFE method. The SAFE extract also contained the highest proportion of aromatic constituents [53.41% (SAFE) vs. 40.92% (DRP-LLE) and 34.47% (DHS)]. The largest proportion of terpenoids was isolated with DHS [19.71% (DHS) vs. 14.56% (DRP-LLE) and 6.43% (SAFE)], while the DRP-LLC extract contained the highest percentage of phenylpropanoids [15.58% (DRP-LLE) vs. 7.41% (SAFE) and 6.61% (DHS)]. As shown in Fig. 1, the major constituents were quite different according to the isolation method. The major constituents isolated from the DRP-LLC method in decreasing order were 4-methoxybenzaldehyde (16.64%), 4-methoxyphenol (10.90%), elemicin (10.62%), *trans*-anethole (10.09%), 4-methoxybenzyl alcohol (7.33%) and carvone (6.45%). The SAFE method yielded the following main compounds: 4-methoxyphenol (23.17%),

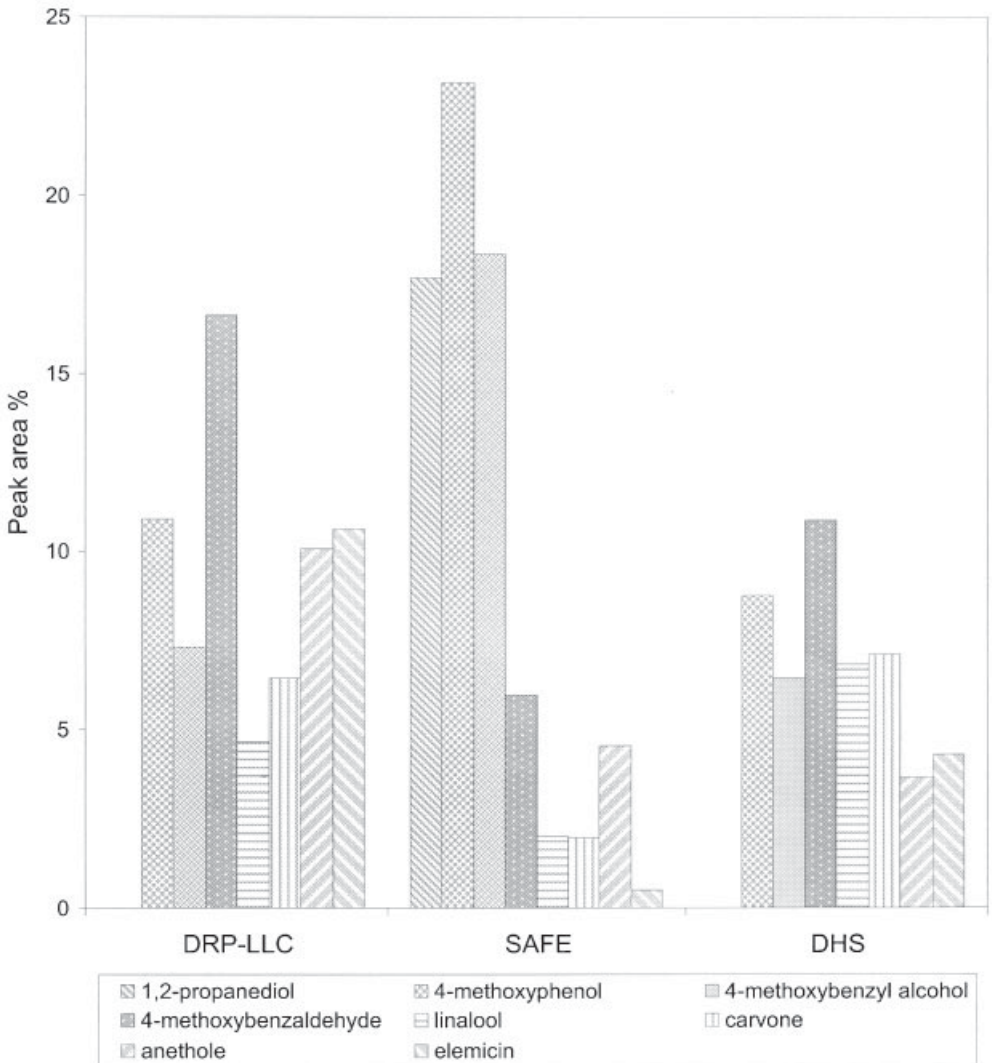

Figure 1. Relative percentage of some major taheebo constituents isolated using three sample preparation methods

4-methoxybenzyl alcohol (18.34%), 1,2-propanediol (17.67%), 4-methoxybenzaldehyde (5.96%) and *trans*-anethole (4.56%). The DHS method recovered 4-methoxybenzaldehyde (10.88%), 4-methoxyphenol (8.77%), carvone (7.12%), linalool (6.84%) and 4-methoxybenzyl alcohol (6.42%) as the major volatiles.

Dichloromethane was used as a solvent in the DRP-LLE and SAFE methods, since it generally appears to be a good solvent for the extraction of a wide range of flavour compounds.[15] One should exercise care with the use of dichloromethane to ensure that traces of HCl have been removed, since this contaminant can lead to artifact formation through acid-catalysed rearrangements.

The volatile extract of taheebo exhibited strong antioxidant activity using two testing systems.[23] In light of previous reports and the chemical structures of components identified in this study, the antioxidant activity of volatile taheebo extract is probably due to the presence of several phenolic components, including 2-methoxy-4-methylphenol, 2-methoxyphenol, 4-methoxyphenol and eugenol. The concentrations of these constituents are sufficiently high to account for the antioxidant activity of taheebo.

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